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Identifying Novel Resistance Genes in Newly Introduced Blast Resistant Rice Germplasm

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ABSTRACT

Blast, *Magnaporthe oryzae* B. Couch, and sheath blight, *Rhizoctonia solani* Kühn, are major fungal diseases of cultivated rice (*Oryza sativa* L.) in the USA. Resistance to U.S. *M. oryzae* races was observed in 91 newly introduced rice accessions, suggesting these accessions are possible sources of novel blast resistance genes (*Pi*-genes) that could be incorporated into U.S. rice cultivars. The genes *Pi-ta* and *Pi-b* have been introduced into U.S. cultivars and characterized molecularly. The objective of this research was to identify new *Pi*-genes in the aforementioned accessions by differentiating known *Pi*-genes, determining relatedness of the accessions with SSR markers, and identifying associations of SSR markers with blast resistance and sheath blight resistance. Twenty-seven accessions were identified with resistance to U.S. blast races and as having neither the *Pi-ta* nor *Pi-b* gene. Based on 125 SSR markers distributed over the rice genome, 11 of the 27 accessions were closely related to each other, but the remaining 16 accessions had varying levels of genotypic diversity, including two accessions selected from crosses of the Asian cultivated species, *O. sativa*, with the African cultivated species, *O. glaberrima*. Blast resistance traits were associated with 32 of the 125 SSR markers and sheath blight resistance traits with 19 markers. Of the 32 blast-associated markers, 20 were located in chromosomal regions previously identified as containing *Pi*-genes. The remaining 12 markers will provide the basis for discovering additional *Pi*-genes.

PLANT breeders often use cultivars developed in other countries to broaden the germplasm base for developing improved cultivars. Dilday (1990) observed that all U.S. rice cultivars developed before 1990 could be traced back to 22 plant introductions that were made into the southern USA (Arkansas, Louisiana, Texas) and 23 introductions made into California. To expand the germplasm base of U.S. cultivars most rice breeding programs are incorporating more diverse rice germplasm into the cultivars being developed (Mackill and McKenzie, 2003). Using 169 SSR markers, Lu et al. (2005) genotyped 145 U.S. rice cultivars and showed cultivars released more recently clustered close to older cultivars in their pedigree. In addition, the cultivars clustered based on their subspecies (tropical japonica, temperate japonica, or indica) and grain type (long, medium, or short grain).

Blast (Couch and Kohn, 2002), causes major rice yield losses worldwide with resistant cultivars and fungicides being the primary methods of disease control (Bonman, 1992; Lee, 1994). Based on the rice genome sequence, Monosi et al. (2004) determined that the rice genome carries approximately 500 nucleotide-binding site (NBS)-leucine-rich repeat (LRR) genes. Many of these NBS-LRR regions are associated with a broad spectrum of disease resistance genes (*R*-genes), as initially reported for the rice blast resistance genes, *Pi-ta* (Bryan et al., 2000) and *Pi-b* (Wang et al., 1999). Monosi et al. (2004) determined the distribution of the NBS-encoding genes in the 'Nipponbare' rice genome and identified the approximate map position of blast resistance (designated *Pi-*) genes, which had been previously mapped to a chromosome region based on phenotype, using various mapping populations. The U.S. rice breeding programs incorporated *Pi-ta* from the Vietnamese landrace Tetep into 'Katy' (Moldenhauer et al., 1990) and *Pi-b* from the Chinese cultivar Te Qing into 'Saber' (McClung et al., 2004). In an effort to utilize molecular markers as part of U.S. breeding programs, SSR markers associated with resistance to *Pi-b*, *Pi-k*^{h}, *Pi-k*^{s}, *Pi-ta*, and *Pi-z* were identified in the background of U.S. cultivars (Conaway-Bormans et al., 2003; Fjellstrom et al., 2004, 2006; Jia et al., 2004b).

Rice sheath blight is another major fungal disease of rice worldwide (Rush and Lee, 1992). Recently, Pinson et al. (2005) confirmed six previously identified QTLs for sheath blight resistance and identified eight new QTLs. Three of the confirmed QTLs were independent for plant height and maturity which affect sheath blight resistance. Based on identification of the aforementioned sheath blight resistance QTLs, additional studies are in progress to further map sheath blight resistance genes.

To characterize additional rice germplasm useful to U.S. rice breeders Dilday et al. (2001), Yan et al. (2002), Lee et al. (2003), and Yan et al. (2003) conducted field evaluations on approximately 1000 rice germplasm accessions recently introduced into the USA. Accessions were evaluated for desirable agronomic characteristics and for resistance to the two major diseases blast and sheath blight. Ninety-one accessions identified as blast resistant in the field tests were selected for further greenhouse tests to determine resistance to blast races commonly found in the USA (Correll et al., 2000). The objective of this research was to identify new blast resistance genes in the 91 selected resistant accessions by (i) differentiating known *Pi*-genes, (ii) determining relatedness of the accessions with SSR markers, and (iii) identifying associations of SSR markers with blast resistance, sheath blight resistance, and/or the morphological traits, plant height and heading date, that affect the expression of sheath blight resistance.

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Abbreviations: Polymerase chain reaction (PCR); Quantitative trait locus (QTL); Simple sequence repeat (SSR); Nucleotide-binding site leucine-rich repeat (NBS-LRR).

MATERIALS AND METHODS

Included in this study were 91 rice accessions recently introduced into the USDA-ARS National Plant Germplasm System, ten U.S. cultivars as controls, Bengal (PI561535), Cocodrie (PI606331), Drew (PI596758), Katy (PI527707), LaGrue (PI568891), Lemont (PI475833), Newbonnet (PI474580), Wells (Moldenhauer et al., 2000), Saber (PI633624), and Zenith (Mackill and McKenzie, 2003). Zenith (CIor7787) is an old cultivar that is currently used as a control in greenhouse blast screening and Te Qing (PI536047) is a cultivar from Guangdong, China. Further information on the accessions and cultivars is available through the USDA-ARS National Plant Germplasm System (http://www.ars-grin.gov/npgs/ ; verified 22 May 2006).

Genomic DNA was extracted from leaf tissue using a CTAB method (Eizenga and Phillips, 1997) or the DNeasy Plant Mini Kit (Qiagen, Valencia, CA) per the manufacturer's instructions. Three dominant markers for the resistant *Pi-ta* allele, one dominant marker for the susceptible pair of *pi-ta* dominant primers (Jia et al., 2002), and one pair of *Pi-b* dominant primers (Fjellstrom et al., 2004) were used to determine the presence of *Pi-ta*, *pi-ta* and *Pi-b*, respectively. The presence or absence of these PCR products was visualized on a 1% agarose gel.

One hundred eighty-three SSR markers were selected from the core set developed and mapped by McCouch et al. (2002). Previously, 169 of these SSR markers were used to genotype 145 U.S. rice cultivars (Lu et al., 2005) and 239 rice accessions from a diverse origin (Garris et al., 2005). Seven of the 125 SSR markers were previously shown to be associated with blast resistance genes *Pi-b* (RM208), *Pi-k*^{h}, *Pi-k*^{s} (RM144, RM224), *Pi-ta* (OSM89) and *Pi-z* (RM3431, RM5963, RM6836) in U.S. germplasm (Conaway-Bormans et al., 2003; Fjellstrom et al., 2004). Very recently, Fjellstrom et al. (2006) developed improved SSR markers for *Pi-z* from rice BAC AP005659. Subsequently, AP5659–1 was used to identify *Pi-z* in this study. The forward primers were labeled with FAM, TET, NED, or HEX fluorescent dyes and the reverse primers were unlabeled. DNA amplifications were performed using an MJ Research PTC-100 96 Plus thermal cycler. PCRs were performed in a 10 μl reaction mix containing 37.5 *n*g of template DNA, 1× PCR buffer, 0.025 unit of *Taq* DNA polymerase (Qiagen, Valencia, CA), 0.2 mmol dNTPs, and 0.8 ρmol of forward and reverse primers. Information on primer sequences and PCR amplification conditions for each set of primers are available at http://www.gramene.org/ ; verified 22 May 2006. PCR products were separated by size using an ABI 3700 DNA analyzer (Applied Biosystems [ABI], Foster City, CA). SSR fragment sizing was performed with Gene Scan® software (ABI) using the Local Southern Method and default analysis settings after which alleles were identified with the Genotyper® software (ABI) and binned manually. For most markers, fractional numbers were rounded to the nearest integer and alleles differing by 1 bp were declared different. SSR data, obtained from genotyping U.S. cultivars with the same ABI 3700 DNA analyzer (Lu et al., 2005), was included for comparison.

One hundred twenty-five markers (Fig. 1) were used for the cluster analysis. The genetic distance matrix developed according to Nei (1972) was used to determine the clusters of genotypes applying the Unweighted Pair Group Method using Arithmetic average (UPGMA) employing NTSYSpc 2.01 (Rohlf, 1997). Calculation of the Polymorphism Information Content (PIC) for each SSR marker is described by Bolstein et al. (1980). The PIC value ascertained the relative value of each marker with respect to the number of alleles at each locus and the relative allele frequency of the alleles in the accessions included in this study.

Selected morphological traits (blast disease ratings taken in the field and greenhouse, sheath blight disease ratings, days to heading and plant height) were extracted from yearly experiment station publications (Dilday et al., 2001; Yan et al., 2002; Lee et al., 2003; Yan et al., 2003; Lee, unpublished data, 2004). Blast inoculations in the field and greenhouse were according to Lee et al. (2003). All reaction to the blast inoculation was on a 0 (no lesions) to 9 (dead) rating scale. Sheath blight inoculation was according to Lee et al. (2003) and the disease was rated on a 0 (no lesions) to 9 (dead) scale. Days to heading is defined as the number of days from emergence to approximately half the plot having panicles emerged at anthesis. Plant height is defined as the distance (cm) between the ground and the tip of the uppermost panicle. The association between the SSR markers and phenotypic traits was calculated using the General Linear Model (GLM) in the TASSEL software (available at http://www.maizegenetics.net (Bioinformatics); verified 9 Jan. 2006) by simple regression. The GLM analysis tests the SSR-trait association between the segregating markers and phenotypes. The associations test a least squares solution (Searle, 1987) to the fixed effects linear model constructed in the GLM.

RESULTS AND DISCUSSION

Identification of Resistant Accessions and Known *R*-genes

Table 1 summarizes data collected on 91 rice accessions initially cataloged as being blast resistant in field tests having leaf blast ratings of 4.5 or lower and the eleven cultivars used for comparison. The aforementioned accessions, selected from approximately 1000 rice accessions being introduced into the U.S. rice germplasm collection, were further evaluated in greenhouse tests to determine resistance to seven blast races that are found in the USA. Four accessions, Aijiaonante, Sheng 10, Zanuo No. 1 and 460 were quickly discovered as being susceptible, having at least two ratings of 5.5 or higher for the individual blast races and thus, were no longer considered desirable sources of new blast resistant genes. The remaining 87 accessions were confirmed as blast resistant.

Dominant markers were used to screen all 91 accessions for presence of the *R*-genes, *Pi-ta* and *Pi-b*, which are the only blast *R*-genes known to have "the perfect markers" based on cloned sequence and publicly available. Both *Pi-ta* and *Pi-b* were identified in 37 of the 91 accessions, with three additional accessions having only *Pi-ta* and 19 accessions having only *Pi-b*. The remaining 32 accessions had neither *Pi-b* nor *Pi-ta* and the blast tests conducted in the greenhouse showed 27 of these 32 accessions had low resistance ratings for the races tested (Table 1). These 27 accessions are possible sources of new blast resistance genes.

Observing each accession's country of origin (Table 1) revealed that 35 of the 39 accessions from the Ivory Coast had *Pi-b*. Twenty-nine of the 35 accessions also had *Pi-ta* and a single accession (Tox 3749–71–1-1–3-2–2) only had *Pi-ta*. By contrast, *Pi-ta* was found in only two of the 36 accessions originating from China whereas, *Pi-b* was identified in ten accessions from China. Twenty-one Chinese accessions had excellent blast resistance and contained neither *Pi-ta* nor *Pi-b*.

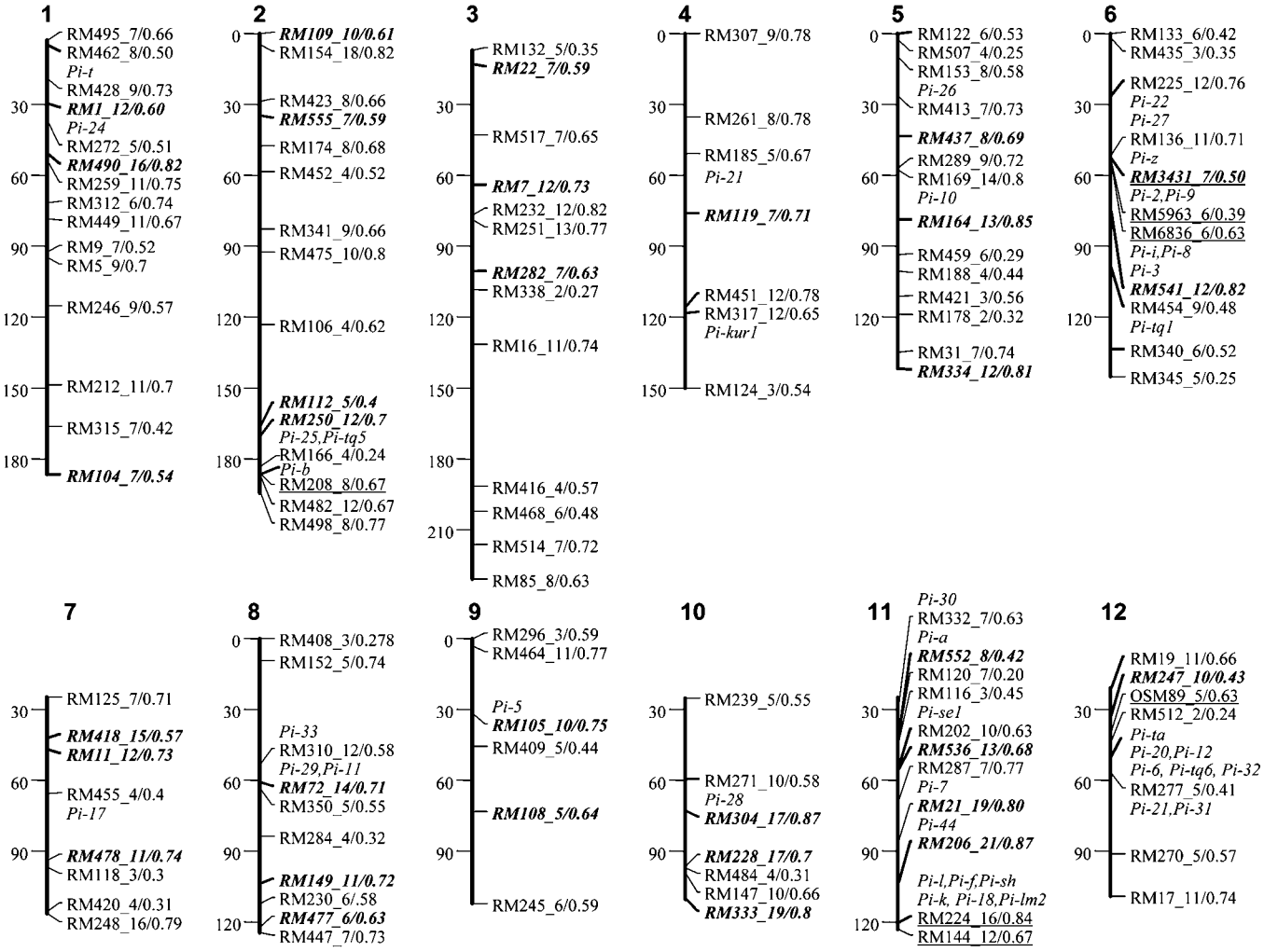

Fig. 1. Diagram illustrating the location in cM of the 125 SSR markers included in this study and the approximate position of known *Pi*-genes as reported by Monosi et al. (2004). Marker positions are based on McCouch et al. (2002). The seven markers used to identify blast resistance genes *Pi-b*, *Pi-k^h*, *Pi-k^s*, *Pi-ta* (Fjellstrom et al., 2004), and *Pi-z* (Conaway-Bormans et al., 2003) in U.S. breeding lines are underlined. Following each SSR marker, the number of alleles associated with each SSR marker used in this study and the PIC value (Bolstein et al., 1980) are listed. Markers identified in this study as associated with blast resistance traits (Table 2) are identified in bold italics. The SSR map was designed with MapChart (Voorrips, 2002).

Subsequent tests with SSR markers for *Pi-z* (Fjellstrom et al., 2006) and *Pi-k* alleles, *Pi-k^s* and *Pi-k^h* (Fjellstrom et al., 2004) indicated three (4642, Iac-47, Wab450–24–3-2-P18-hb) of the 27 accessions had one of these alleles (Table 1). Considering that (i) neither *Pi-z* nor *Pi-k* have the broad spectrum of resistance to U.S. blast races that is found in *Pi-ta* and *Pi-b* (Fjellstrom et al., 2004) and (ii) these three accessions were resistant to all U.S. blast races tested, there are additional *Pi*-genes present besides the possible *Pi-z* or *Pi-k* gene.

In addition to the leaf blast rating, the field data collected on the 91 accessions included sheath blight resistance and the agronomic characters, days to heading and plant height as shown in Table 1. Sheath blight resistance ratings ranged from 3.3 to 8.0 with 29 accessions rated between 3.3 and 5.0, indicating moderate field resistance to sheath blight. Because of the interaction between sheath blight resistance and later heading date (Pinson et al., 2005), it was determined that 20 of the 29 more resistant accessions took 95–110 d to heading as compared to less than 85 d for U.S. cultivars. Comparing sheath blight resistance ratings and plant height revealed 18 of the 29 resistant accessions ranged in height from 100–129 cm as compared to 100 cm or less for the more recent U.S. cultivars. Of the original 29 sheath blight resistant accessions, only three accessions, 4484, 46411 and Let 3137, did not have either a later heading date and/or a taller plant height.

SSR markers Identify the Diverse Background of the Resistant Accessions

To identify different novel *R*-genes it is essential to know how these resistant accessions are related to each other. Most likely different novel *R*-genes will be present in accessions that do not cluster closely together. A total of 125 SSR markers were used to genotype the 91 accessions and 11 cultivars used as controls. The distribution of the 125 SSR markers is shown in Fig. 1 along with the number of alleles and PIC value for the

Table 1. Rice accessions rated for blast and sheath blight resistance, days to 50% heading and plant height. Disease ratings are based on a 0 (no disease) to 9 (dead) scale. Presence of the blast resistance alleles *Pi-ta* and *Pi-b* was determined using PCR-based markers. Presence of *Pi-k* and *Pi-z* alleles was based on specific alleles identified with SSR markers.

			M. oryzae race resistance rating (Isolates)‡											Marker present¶			
No.	Accession name	Country of origin†	IB-1 (ZN-15)	IB-49 (ZN-52)	IC-17 (ZN-1)	IE-1 (ZN-6)	IE-1K (ZN-19)	IG-1 (ZN-39)	IH-1 (74L2)	Leaf blast in field§	Sheath blight resistance§	Days to heading§	Plant height (cm)§	*Pi-b*	*Pi-ta*	*Pi-k*	*Pi-z*
1	Bhujon Kolpo	Bangladesh	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	4.3	100	90	yes	no	no	no
2	Bogra	Bangladesh	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.8	5.7	90	104	yes	no	no	no
3	Khoia	Bangladesh	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	5.7	89	106	yes	yes	no	no
4	Iac 47#	Brazil	1.5	0.5	2.0	0.0	1.0	0.0	0.0	2.5	5.5	81	129	no	no	k^h	no
5	IRGA409	Brazil	0.0	0.0	0.0	0.3	0.5	0.0	0.0	1.0	5.0	94	101	yes	no	no	no
6	Guang-6ai-4#	China	1.5	1.3	2.0	0.0	0.0	0.0	1.0	–	–	72	87	no	no	no	no
7	02428#	China-CD	1.7	0.0	0.3	0.0	0.5	1.5	0.0	0.0	5.8	84	124	no	no	no	no
8	Chunzhi No. 11#	China-CD	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.0	85	108	no	no	no	no
9	Fu No83	China-CD	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	5.5	90	99	yes	no	no	no
10	Kechengnuo No. 4#	China-CD	0.2	0.0	0.0	–	0.3	0.0	0.0	0.0	6.0	90	106	no	no	no	no
11	Sheng 10	China-CD	5.5	6.8	5.8	4.0	4.0	5.5	4.3	4.5	7.0	73	115	yes	no	no	no
12	Shufeng 117#	China-CD	0.0	0.0	0.0	0.0	0.0	0.3	0.3	0.0	5.3	91	97	no	no	no	no
13	Shufeng 122#	China-CD	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	5.3	97	94	no	no	no	no
14	Tie 90–1#	China-CD	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.8	72	87	no	no	no	no
15	Tiejing No. 4#	China-CD	0.3	0.7	0.3	0.0	1.3	3.0	0.0	0.0	7.0	64	90	no	no	no	no
16	Zhang 32	China-CD	4.3	6.0	0.3	0.0	1.3	2.2	6.5	0.0	6.0	64	92	yes	no	no	no
17	Xiangzaoxian No. 1#	China-HN	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	5.3	64	78	no	no	no	no
18	71198	China-HZ	0.0	1.0	0.3	0.0	0.0	0.0	0.5	0.0	6.3	84	94	yes	no	no	no
19	Aijiaonante	China-HZ	5.7	6.7	6.0	2.0	0.3	5.0	6.7	0.0	7.0	72	80	no	no	no	no
20	Zanuo No. 1	China-HZ	4.3	6.0	6.3	–	0.0	1.7	–	0.0	5.5	69	90	no	no	no	no
21	Zhongyu No. 1#	China-HZ	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.8	83	85	no	no	no	no
22	Zhongyu No. 6	China-HZ	0.0	0.3	1.0	0.0	0.0	0.0	0.0	0.0	5.8	90	90	yes	no	no	no
23	Zhongzao No. 1	China-HZ	2.7	6.3	0.3	0.0	1.0	3.5	4.3	0.0	6.8	66	80	no	no	no	no
24	460	China-JT	1.0	6.0	7.0	0.0	4.0	7.3	0.0	0.0	5.5	83	104	no	no	no	no
25	2410	China-JT	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	6.8	73	90	yes	yes	no	no
26	4484	China-JT	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.0	81	92	yes††	no	no	no
27	4593#	China-JT	0.3	0.0	0.3	0.0	0.0	0.0	0.0	0.0	5.5	89	92	no	no	no	no
28	4594#	China-JT	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.0	87	94	no	no	no	no
29	4596#	China-JT	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.0	89	87	no	no	no	no
30	4597#	China-JT	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.3	90	92	no	no	no	no
31	4607#	China-JT	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	5.5	90	90	no	no	no	no
32	4611#	China-JT	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	6.0	89	92	no	no	no	no
33	4612#	China-JT	0.2	0.0	0.0	0.0	0.0	0.3	0.0	0.0	5.8	90	90	no	no	no	no
34	4632#	China-JT	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.5	88	87	no	no	no	no
35	4633#	China-JT	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.0	89	92	no	no	no	no
36	4642#	China-JT	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	5.5	90	85	no	no	no	yes
37	4641(1)#	China-JT	0.7	0.3	0.0	0.0	0.0	3.5	0.0	0.0	4.8	90	90	no	no	no	no
38	GP-2	China-JT	1.0	2.0	0.0	0.0	3.0	0.0	0.0	1.8	5.8	89	99	yes	yes	no	no
39	Gui 99	China-JT	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.5	90	97	yes	no	no	no
40	R 147	China-JT	0.0	0.0	6.0	0.0	0.0	1.0	0.0	0.0	5.8	81	90	no	no	no	no
41	R 312#	China-JT	0.3	0.0	0.3	0.0	0.0	0.0	0.0	0.0	6.3	90	92	no	no	no	no
42	Dian No. 1	China-KM	0.0	0.3	0.0	0.0	0.0	0.0	1.8	0.0	5.8	80	101	yes	no	no	no
43	Egyptian Jasmine	Egypt	0.0	0.0	0.3	0.0	0.0	0.3	0.3	4.0	4.3	95	99	yes††	yes‡‡	no	no
44	GZ-1368–5-4	Egypt	0.0	0.3	0.0	0.0	0.0	0.0	1.0	1.8	5.3	85	106	yes	yes	no	no
45	GZ-5578–2-1–2	Egypt	0.0	0.0	0.0	0.0	1.8	0.0	0.0	0.8	7.3	79	92	no	yes	k^s	yes
46	GZ-5594–23–1-2	Egypt	0.0	0.0	0.0	0.0	2.3	0.0	0.0	3.0	6.0	81	90	yes	yes	no	no
47	GZ-5830–48–2-2	Egypt	0.0	0.0	0.5	0.0	0.0	0.0	0.0	1.8	7.3	81	99	yes	no	k^s	no
48	Ad 9246	Ivory Coast	0.0	0.3	0.0	0.0	0.0	0.0	0.0	1.0	5.1	94	107	yes	yes	no	no
49	Fkr 19 (Tox728–8)#	Ivory Coast	0.5	0.0	0.0	0.0	0.0	1.0	0.5	2.3	5.2	87	130	no	no	no	no
50	Fkr 48	Ivory Coast	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.3	4.2	101	124	yes	yes	no	no
51	32 Xan Sc	Ivory Coast	1.0	0.3	0.0	0.0	0.0	0.0	0.0	1.3	4.8	90	113	yes††	no	no	no
52	Ita 406	Ivory Coast	0.3	0.0	0.0	0.0	0.0	0.0	0.5	0.8	4.0	101	104	yes	yes	no	no
53	Ita 416	Ivory Coast	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	5.0	84	120	yes	yes	no	no
54	Let 3137	Ivory Coast	0.0	0.0	0.5	0.0	1.0	0.0	0.0	0.8	5.0	91	94	yes	no	no	no
55	S 992-F4–2-5–1-B	Ivory Coast	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3	5.3	89	94	yes	yes	no	no

56	Tnau 7893	Ivory Coast	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	6.0	86	99	yes	yes	no	no
57	Tox 3093–35–2-3–3-1	Ivory Coast	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.8	5.0	99	94	yes	yes	no	no
58	Tox 3211–49–1-1–3-2	Ivory Coast	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	4.3	108	101	yes	yes‡‡	no	no
59	Tox 3241–21–2-2–3-2	Ivory Coast	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	4.7	104	99	yes	yes	no	no
60	Tox 3241–22–3-3–3	Ivory Coast	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	4.0	104	99	yes	no‡‡	no	no
61	Tox 3241–31–2-1–3-1	Ivory Coast	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	4.3	103	101	yes	no	no	no
62	Tox 3441–123–2-3–2-2–2	Ivory Coast	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	5.0	100	104	yes	no	no	no
63	Tox 3553–34–3-2–3-2–2	Ivory Coast	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	5.0	96	106	yes††	yes	no	no
64	Tox 3706–60–3-3–3	Ivory Coast	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.5	85	97	yes	yes	no	no
65	Tox 3706–6-3–3-2	Ivory Coast	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.8	4.3	90	110	yes	yes	no	no
66	Tox 3716–4-3–2-2–2-2	Ivory Coast	0.0	0.3	0.3	0.0	0.3	0.0	0.0	0.5	5.3	87	101	yes	yes	no	no
67	Tox 3717–25–3-1–3	Ivory Coast	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	4.0	99	99	yes	yes	no	no
68	Tox 3717–25–3-3–1	Ivory Coast	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	5.1	103	90	yes	yes	no	no
69	Tox 3717–25–3-3–2	Ivory Coast	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	3.8	100	94	yes	yes	no	no
70	Tox 3717–76–2-2–3	Ivory Coast	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.7	88	106	yes	yes	no	no
71	Tox 3717–81–1-1–3	Ivory Coast	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	5.0	90	106	yes	no	no	no
72	Tox 3770–17–2-2–1	Ivory Coast	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	4.5	105	99	yes	yes	no	no
73	Tox 3771–144–2-1–1	Ivory Coast	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	4.0	110	104	yes	yes	no	no
74	Tox 3772–38–2-2–3	Ivory Coast	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	5.3	89	92	yes	yes	no	no
75	Tox 3772–40–3-2–2	Ivory Coast	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.5	4.7	101	104	yes	yes	no	no
76	Tox 3772–94–1-1–1	Ivory Coast	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.5	4.0	103	101	yes	yes	no	no
77	Tox 3779–51–2-2–2	Ivory Coast	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	5.5	98	99	yes	yes	no	no
78	Tox 3867–19–1-1–3-1–1-1	Ivory Coast	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.5	4.0	107	101	yes	yes‡‡	no	no
79	Tox 3869–34–1-3–1-1–3-3	Ivory Coast	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	5.5	99	110	yes	yes	no	no
80	Tox 3872–61–3-3–3-2–1	Ivory Coast	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	3.3	110	104	yes	yes	no	no
81	Tox 3894–41–2-3–1	Ivory Coast	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	4.0	89	108	yes	yes‡‡	no	no
82	Tox 4136–38–2	Ivory Coast	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3	5.3	89	101	yes	yes	no	no
83	Tox 4251–313–3	Ivory Coast	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	3.7	103	112	yes	yes	no	no
84	Tox 3749–71–1-1–3-2–2	Ivory Coast	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.8	5.0	106	108	no	no‡‡	no	no
85	Wab450–24–3-2-P18-hb#	Ivory Coast	0.3	1.0	0.3	0.3	0.0	0.3	0.0	0.0	6.3	77	110	no	no	k^h	no
86	Wab450-I-B-P-62-hb#	Ivory Coast	0.5	2.5	0.0	1.0	0.0	1.8	0.0	0.8	7.3	77	104	no	no	no	no
87	Pyongyang 23	Korea, N.	0.8	0.3	1.0	1.0	0.0	1.0	6.3	0.7	6.3	85	101	no	no‡‡	k^s	no
88	IR56450–28–2-2 1	Philippines	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.5	4.8	89	104	yes	yes	no	no
89	Nj70507 17578#	Philippines	0.0	0.0	1.0	1.0	0.0	0.0	0.0	1.0	5.7	91	115	no	no	no	no
90	RP2199–16–2-2–1	Philippines	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.7	5.2	90	113	yes	yes‡‡	no	no
91	S972B-22–1-3–1-1	Philippines	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.5	5.8	88	97	yes	no	no	no
92	Bengal	USA	6.0	6.8	2.0	1.3	0.5	0.5	0.0	6.3	7.3	82	71	no	no	k^s	yes
93	Cocodrie	USA	5.0	1.8	6.8	5.5	0.3	1.0	0.0	0.8	7.0	76	76	no	no	k^h	no
94	Drew	USA	0.0	0.0	0.0	0.0	6.8	0.0	0.0	0.8	7.5	77	90	no	yes	k^s	no
95	Katy	USA	0.0	0.5	0.0	0.0	7.0	0.0	0.0	7.0	6.5	78	99	no	yes	no	no
96	LaGrue	USA	6.8	7.8	7.5	6.0	5.8	5.5	8.0	6.5	6.8	82	104	no	no	no	no
97	Lemont	USA	7.0	7.3	6.5	7.8	7.3	1.0	0.0	2.0	4.3	82	78	no	no	k^h	no
98	Newbonnet	USA	7.0	7.8	0.3	6.0	5.3	2.3	0.3	7.8	7.5	83	108	no	no	k^h	no
99	Saber	USA	2.5	5.0	3.0	1.0	1.3	0.3	0.0	1.0	6.3	90	102	yes	no	k^h	no
100	Te Qing	China/USA	0.0	0.0	0.0	–	–	0.0	1.0	1.0	4.5	96	97	yes	yes	no	no
102	Wells	USA	6.8	7.3	7.8	4.8	4.5	0.0	0.3	5.7	6.8	76	80	no	no	k^h	no
103	Zenith	USA	5.8	6.0	2.8	0.3	6.8	0.5	0.5	–	–	82	143	no	no	no	yes

† Source of Chinese germplasm: CD-Chengdu, HN-Hunan, HZ-Hongzhou, KM-Kunming, JT-Joshua Tao.

‡ Race identification according to Correll et al. (2000). Data from Yan et al. (2002), Lee et al. (2003) and Lee (personal communication, 2004).

§ Data for field leaf blast ratings, field sheath blight ratings, days to 50% heading and plant height extracted from Dilday et al. (2001), Yan et al. (2002), Lee et al. (2003), Yan et al. (2003) and Lee (personal communication, 2004).

¶ Indicates the visualization of *Pi-ta* and/or *Pi-b* dominant markers on an agarose gel. The presence of two *Pi-k* alleles, k^s or k^h, and *Pi-z* are based the polymorphism of the SSR markers RM144 and RM224 for *Pi-k* (Fjellstrom et al., 2004), and AP5659–1 for *Pi-z* (Fjellstrom et al., 2006).

Accessions that are possible sources of new/novel blast resistance genes.

†† The SSR marker (RM208) polymorphism for presence of *Pi-b* does not agree with the dominant marker visualization.

‡‡ Analysis using the single nucleotide length polymorphism marker for *Pi-ta* (Jia et al., 2004a) indicated this locus was heterozygous for the *Pi-ta* and *pi-ta* alleles associated with resistance and susceptibly, respectively.

individual markers. The number of alleles present in these 125 markers ranged from 2 to 21 with 64 markers having eight or more alleles associated with them. The PIC values ranged from 0.208 to 0.870 with 73 markers having values greater than 0.700. These values indicate there is adequate polymorphism for the 125 markers tested.

Using Nei's genetic distance determination, the 91 accessions and 11 cultivars divided into two main clusters designated I and II (Fig. 2). The smaller cluster (I) divided into the U.S. cultivars (I-A) included for comparison, which divided into medium grain (Bengal, Zenith) and long grain types (all others). Clustered with the U.S. cultivars was a sub-cluster (I-B) containing a) two accessions, Wab450–1-B-P-62-hb and Wab450–24–3-2-P18-hb, derived from crosses of the Asian cultivated species, *O. sativa*, with the African cultivated species *O. glaberrima* Steud., b) three Egyptian accessions, GZ-5830–48–2-2, GZ-5578–2-1–2 and GZ-5594–23–1-2, and c) one each of accessions from Chengdu, China (02428); 460 donated by J. Tao from China; IAC47 developed in Brazil; and the single North Korean accession, Pyongyang 23. The cluster analysis indicated that these accessions were developed from a similar parentage.

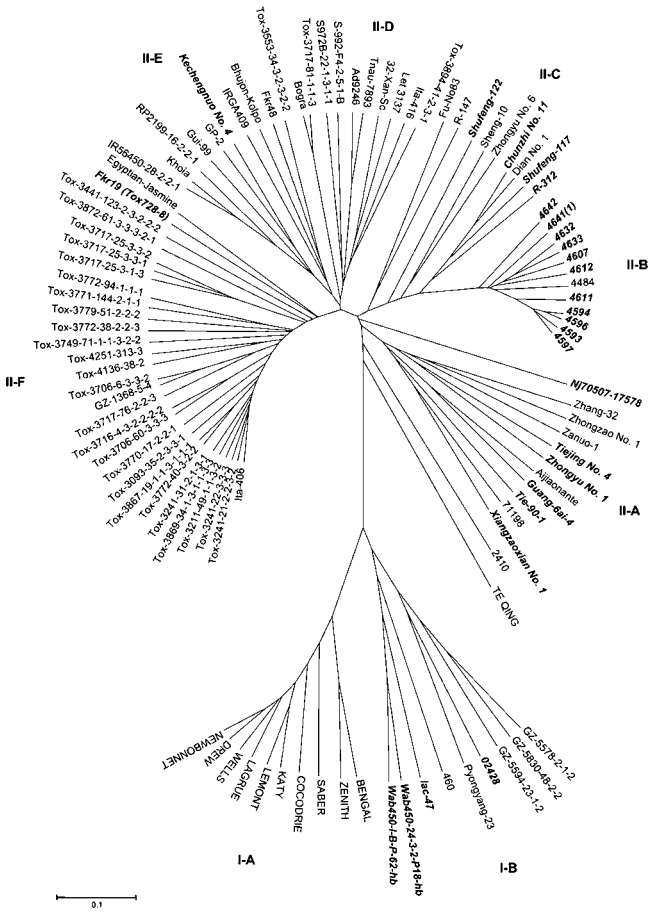

Fig. 2. Genetic distance among 91 blast resistant rice accessions and eleven controls (capitalized) as revealed by UPGMA cluster analysis and Nei (1972). The two main clusters are identified as I and II with sub-clusters denoted with letters (A-F). The controls included ten selected U.S. rice cultivars and Te Qing, which originated in China and is used as a parent in some U.S. breeding programs. Accessions in bold italics were identified as resistant to all blast isolates tested and not having one of the *R*-genes, *Pi-b* and/or *Pi-ta*.

The other main cluster (II) had sub-clusters of the accessions obtained from various sources in China and the Ivory Coast. Te Qing, which originated from Guangdong, China and is being used as a parent in U.S. breeding programs, was distantly clustered in this group. Other accessions originating from China which cluster together include the following sub-clusters: II-B with twelve accessions donated to the U.S. rice germplasm collection from one source (J. Tao); II-A with five accessions from Hongzhou, three from Chengdu, one from Hunan and Guang-6ai-4; and II-C with accessions from several regions Chengdu, Hongzhou, Kumming and J. Tao. The presence of these sub-clusters suggests several of these accessions have a similar parentage. Accessions obtained from the Ivory Coast subdivided into three sub-clusters (II-D, II-E, II-F). The largest cluster (II-F) included 27 accessions with three accessions [Fkr19 (Tox728–8), Tox3441–123–2-3–2-2–2, Tox3872–61–3-3–3-2–1] being more distant. The accessions that cluster closer most likely have the same parentage and those clustering more distant have one parent that was different. Sub-cluster II-D included eight accessions from the Ivory Coast, Bogra from Bangladesh, and S972B-22–1-3–1-1 from the Philippines. The remaining sub-cluster (II-E) of ten accessions included Khoia and Bhujon-Kolpo from Bangladesh; IRGA 409 from Brazil; IR56450–28–2-2–1 and RP2199–16–2-2–1 from the Philippines; Fkr48 and Tox3553–34–3-2–3-2–2 from the Ivory Coast; and Gui-99, GP-2, and Kechengnuo-4 from China. The fact that these ten accessions originated from five different countries representing three different continents, suggests some common parentage in the background of the accessions.

Figure 2 also denotes (bold, italics) the aforementioned 27 accessions identified as blast resistant and having neither *Pi-b* nor *Pi-ta*. Based on the genetic distance determination, eleven of the 27 accessions were clustered closely together and were obtained from one source (J. Tao). The remaining 16 accessions were divided among five large clusters indicating these accessions had at least five different backgrounds thus, it is likely that different novel *R*-genes are present in these accessions.

SSR Markers Associated with Blast and Sheath Blight

The first association mapping conducted in plants associated the flowering time of 92 inbred maize lines with polymorphisms in the *Dwarf8* gene using 141 SSR markers (Thornsberry et al., 2001). Following similar methods, associations were calculated for each individual SSR marker with leaf blast field ratings, the seven individual blast race ratings, sheath blight ratings, plant height and days to heading (Table 2). Forty of the 125 SSR markers had significant associations with at least one of the aforementioned traits, and one marker, RM021, had eight traits associated with it. Thirty-three of the 40 markers were associated with at least one blast

Table 2. Significant SSR-trait associations of 40 individual SSR markers with blast race ratings, field leaf blast ratings, sheath blight ratings, plant height, and days to heading determined over the 91 accessions and eleven cultivars using simple regression in TASSEL (http://www.maizegenetics.net (Bioinformatics); verified 22 May 2006).

			M. oryzae race identification (Isolates)‡										
SSR marker†	Chromosome	Position (cM)	IB-1 (ZN-15)	IB-49 (ZN-52)	IC-17 (ZN-1)	IE-1 (ZN-6)	IE-1K (ZN-19)	IG-1 (ZN-39)	IH-1 (74L2)	Leaf blast in field	Sheath blight resistance	Plant height	Days to heading
RM001	1	29.7	0.52										
RM490	1	51.0			0.53	0.56		0.48			0.41		
RM104	1	186.6					0.80						
RM109	2	0.0								0.55			
RM154	2	4.8										0.57	
RM555	2	34.7	0.52				0.69			0.54			
RM341	2	82.7									0.43		
RM112	2	166.0					0.66			0.50			
RM250	2	170.1	0.56				0.77			0.55			
RM022	3	13.0					0.67						
RM007	3	64.0	0.53	0.45			0.79			0.61			
RM251	3	79.1									0.40		
RM282	3	100.6					0.70						
RM119	4	76.1								0.65			
RM413	5	26.7									0.43		0.45
RM437	5	43.4	0.51										
RM164	5	78.7					0.69						
RM334	5	141.8				0.71							
RM435	6	2.2									0.43		
RM3431	6	53.0					0.75	0.47					
RM541	6	75.5	0.53		0.42	0.58				0.50	0.55	0.52	
RM418	7	42.1	0.65	0.60					0.57				
RM011	7	47.0	0.53	0.52	0.40		0.66						
RM478	7	93.8				0.67	0.67			0.69			
RM248	7	116.6									0.51		0.48
RM072	8	60.9					0.76			0.58	0.44		
RM149	8	103.7					0.79			0.57			0.45
RM477	8	121.8	0.62	0.54	0.44		0.68				0.53		0.50
RM464	9	3.3											0.47
RM105	9	32.1							0.40		0.48		0.52
RM108	9	73.3						0.40					
RM304	10	73.0							0.72				0.44
RM228	10	96.3						0.42					
RM333	10	110.4		0.53	0.57			0.60	0.52		0.43		0.52
RM552	11	40.6	0.63	0.49		0.74	0.82			0.57		0.44	
RM536	11	55.1	0.61	0.54	0.43	0.59	0.65			0.50			
RM021	11	85.7	0.51	0.52	0.46	0.63	0.81	0.46		0.69	0.46		
RM206	11	102.9			0.48			0.56			0.49		
RM144	11	123.2										0.43	
RM247	12	32.3			0.48	0.60							

† Additional information on the SSR markers for the accessions included in this study are available from the corresponding author.
‡ Race identification according to Correll et al. (2000).

trait. The range in the number of markers associated with the individual blast races was from four markers associated with IH-1 to 17 markers with IE-1K. Thirteen markers were associated with field ratings for leaf blast and 13 with sheath blight. With traits that influence sheath blight resistance, plant height, and days to heading, eight markers were associated with plant height and four with days to heading. Five markers, RM105, RM248, RM333, RM413, and RM477, were associated with both sheath blight and days to heading, and one marker, RM541, with both sheath blight and plant height. Chromosome 11 had the most, five markers associated with nine of the 11 phenotypic traits evaluated. Four of the five markers were associated with blast resistance traits.

In Fig. 1 the SSR markers that were associated with at least one of the blast traits measured are italicized, and the approximate map location of reported blast resistance (*Pi-*) genes, as summarized by Monosi et al. (2004), are marked. The association mapping we performed identified valid blast resistance gene locations as evidenced by the fact that of the 32 markers associated with blast traits, 20 markers were located in regions previously reported to contain one or more *Pi*-gene(s) and/or QTLs for blast (Ramalingam et al., 2003). Three (RM22, RM104, RM282) of these 20 markers were only located in regions of the blast QTLs. In addition, seven (RM11, RM108, RM109, RM149, RM418, RM477, RM555) of the remaining twelve markers are located in the genetic map positions of NBS-LRR identified by Monosi et al. (2004). The remaining five markers (RM7, RM228, RM333, RM334, RM478) identified by association mapping were not in regions previously identified with blast traits and/or NBS-LRR sites.

Of the seven SSR markers discovered by Fjellstrom et al. (2004) to identify blast genes *Pi-b* (RM208), *Pi-k* (RM144, RM224), *Pi-ta* (OSM89), and Conaway-Bormans et al. (2003) to identify *Pi-z* (RM3431, RM5963, RM6836) in the background of U.S. breeding lines, only one (RM3431) was associated with blast traits in this study using more diverse germplasm. RM3431, one of three markers for *Pi-z*, was associated with resistance to blast races IG-1 and IE-1K. Previous studies show that *Pi-z* confers resistance to both of these blast races

(Conaway-Bormans et al., 2003). The lack of association with RM208 and OSM89, even though dominant primers for the cloned genes suggest *Pi-b* is present in 56 accessions and *Pi-ta* in 40 accessions, is most likely due to these markers being identified in southern U.S. rice breeding lines and not the diverse background of these accessions (Fjellstrom et al., 2004; Jia et al., 2004b). Lack of association with RM144 and RM224 may be due to few accessions having the *Pi-k*^{h} or *Pi-k*^{s} alleles and/or the influence of the diverse background. The distance from the *Pi-z* locus, diverse background, or an altered *Pi-z* locus, may explain why only RM3431 showed an association. According to Fjellstrom et al. (2006), the original *Pi-z* markers, RM3431, RM5963, RM6836, are not close enough to the locus and have not always worked well for U.S. breeding programs thus, they recently developed new SSR markers for *Pi-z* from the rice BAC AP005659.

Early studies of *Pi-k* indicated a large number of alleles present at this locus (Kiyosawa, 1972; McCouch et al., 1994). More recently, studies of *Pi-b* (Fjellstrom et al., 2004), *Pi-ta* (Jia et al., 2004b) and *Pi-z* (Hayashi et al., 2004, Fjellstrom et al., 2006) suggest multiple alleles may also be present at these loci based on gene sequence information, mapping populations and reaction to blast isolates. In addition to affecting the association identified, slightly altered alleles also may explain why the presence/absence of *Pi-b* as determined with RM208 and the *Pi-b* dominant primer results disagreed for four accessions (Table 1).

The location of SSR markers associated with sheath blight resistance, days to heading, or plant height were compared to recently summarized QTL maps for these traits (Pinson et al., 2005; Zou et al., 2000). Based on Pinson et al. (2005; S.R. Pinson, personal communication, 2005) of the 13 markers associated with sheath blight resistance (Table 2), four (RM72, RM413, RM477, RM490) have already been identified as sheath blight resistance QTLs, seven (RM341, RM251, RM435, RM541, RM105, RM33) are near sheath blight resistance QTLs, and the remaining three markers (RM248, RM21, RM206) were not close to any previously reported sheath blight resistance QTL. Of the four markers associated with plant height, RM541 and RM552 were near previously located sheath blight resistance QTLs (Pinson et al., 2005; S.R. Pinson, personal communication, 2005); RM144 was in the same region as RM206, identified in this study as associated with sheath blight; and RM154 was not related to previously reported QTLs for sheath blight resistance or plant height. Eight markers were associated with days to heading. Comparing these associations to studies by Pinson et al. (2005; personal communication, 2005), RM464 was previously associated with late heading and a sheath blight resistance QTL; RM413 and RM477 were used as markers for sheath blight resistance QTLs; and RM149, RM105, RM304 and RM333 were located near sheath blight resistance QTLs. RM248 was identified in this study as associated with sheath blight resistance, but not in Pinson et al. (2005) or Zou et al. (2000). In summary, of the 19 SSR markers associated with sheath blight resistance, heading date, or plant height, 16 were in regions previously identified in other QTL mapping studies, indicating that most of the associations identified are supported by inheritance studies of these traits.

In conclusion, from the 91 rice germplasm accessions selected from approximately 1000 accessions, 27 accessions were identified as resistant to U.S. blast races that did not have the blast resistance genes, *Pi-ta* or *Pi-b*, already incorporated into U.S. rice cultivars. Eleven of the 27 accessions were closely related to each other but the remaining 16 accessions had varying levels of genotypic diversity, including two accessions selected from crosses with *O. glaberrima*, one from the Philippines and one from the Ivory Coast. Thirty-two SSR markers were associated with blast resistance traits and 19 with sheath blight resistance traits. The fact that 27 of the markers associated with blast resistance traits and 17 of the markers associated with sheath blight resistance were located in regions identified in previously published inheritance studies, strengthens the validity of our association mapping results. This documented support provides encouragement that the remaining markers may prove relevant for identifying the additional resistance genes necessary for U.S. rice breeding programs.

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